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A novel validated quantitative estimation & stability indicating RP-HPLC method for simultaneous estimation of tolperisone HCL and etodolac in bulk and its pharmaceutical dosage formulations

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ABSTRACT

A rapid, sensitive and specified RP-HPLC method involving DAD detection was developed and validated for determination and quantification of Tolperisone (TOL) and Etodolac (ETD) in tablet dosage form. Chromatography was carried out on Sunsil, 250 mm x 4.6 mm i.d; 5µ particle size column using filtered and degassed mixture of Acetonitrile and phosphate buffer (pH 2.6) in the ratio of 30:70 v/v as mobile phase at a flow rate of 1 ml/min and effluents were monitored at 267 nm. The pH of the mobile phase was adjusted with the diluent. The method was validated in terms of linearity, precision, accuracy, specificity, Limit of detection, limit of quantification and stability indicating studies. The assay was linear over the concentration range for TOL: 18-42µg/mL and for ETD: 48-112 µg/mL respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drugs to the pre analysed test solutions and was found to be between 99.27 – 101.38% within. The % RSD for both TOL & ETD was found to be 0.66 and 0.41 respectively. The stability indicating studies were all in limit and meeting the need of the quantitative analysis. The method does require less than 10 minutes as run time for analysis which enhanced to prove the adoptability of the method for routine quality control of the drugs.

Keywords: Tolperisone (TOL) and Etodolac (ETD), Estimation, Validation and Stability studies.

INTRODUCTION

Tolperisone (TOL), chemically (R, S) 2-methyl-1-(4-methyl phenyl)-3- (1-piperidyl) propan-1one**,** Methyl derivative, piperidyl ring, carbonyl derivative, chlorine moiety, Tolperisone is indicated for use in the treatment of pathologically increased tone of the cross-striated muscle caused by neurological diseases (damage of the pyramidal tract, multiplesclerosis, myelopathy, encephalomye litis) and of spastic paralysis and other encephalopathies manifested with muscular dystonia [1, 2].

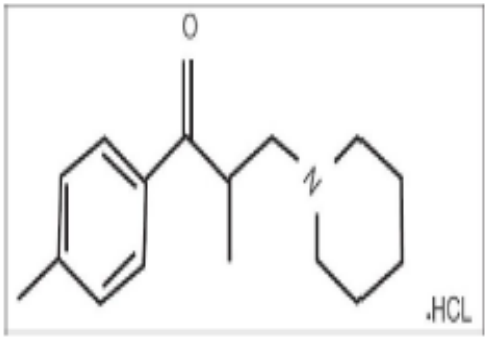

Figure 1: structure of Tolperisone

Etodolac (ETD) chemically (RS)2—(1,8-Diethyl-4,9-dihydro-3H-pyrano[3,4-b]indo-1-yl)acetic acidIndole ring derivative, pyranosyl derivative, carbonyl group, acetate moiety Indole ring derivative, pyranosyl derivative, carbonyl group, acetate moieties. Etodolac (ETD) is a nonsteroidal anti-inflammatory drug (NSAID). NSAIDs are used for the management of mild to moderate pain, fever, and inflammation. They work by reducing the levels of prostaglandins, which are chemicals that are responsible for pain and the fever and tenderness that occur with inflammation. Etodolac blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower concentrations of prostaglandins [3, 4,5]. As a consequence, inflammation, pain and fever are reduced. Post-marketing studies demonstrated that Etodolac inhibition of cyclooxygenase is somewhat COX-2 selective [6] similar to celecoxib and other "COX-2 inhibitors." Unlike rofecoxib, both Etodolac and celecoxib can fully inhibit COX-1 and are designated as having "preferential selectivity" toward COX-2. The (inactive against COX) r-enantiomer of Etodolac inhibits β-catenin levels in hepatomacells [7].

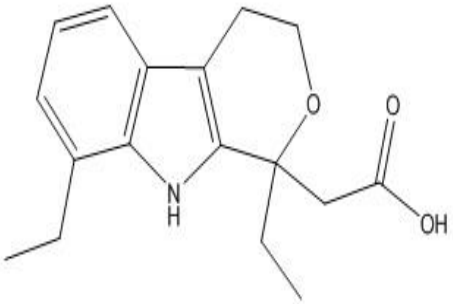

Figure 2: Structure of Etodolac

MATERIALS AND INSTRUMENTATION

Drugs

Tolperisone and Etodolac gift samples were procured from Chandra Labs, Hyderabad.

Chemicals & Reagents Used

HPLC grade Acetonitrile (Merck specialities Private Limited, Mumbai)

Sodium Hydroxide (Merck specialities Private Limited, Mumbai)

Laboratory grade Potassium Dihydrogen phosphate (Merck chemicals limited)

HPLC grade Double distilled water (Merck specialities Private Limited, Mumbai)

Laboratory grade ortho phosphoric acid, (SD Fine Chemicals, Mumbai)

All dilutions were performed in standard class-A, volumetric glassware.(Borosil)

Instrumentation

Agilent 1120 compact LC chromatographic system, with DAD detector and a fixed injector equipped with 20 µL loop was used for the

chromatographic separation. The chromatogram was recorded at and peaks quantified by means of Ezchrome software. Chromatographic separation was carried out on a C_{18} column [Sunsil, 250 mm x4.6 mm 5µ particle size]. Sartorius electronic balance was used for weighing the samples. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

Chromatographic separation of Tolperisone and Etodolac was carried on a C_{18} column. The mobile phase was composed of acetonitrile and phosphate buffer (pH 2.6) in the ratio of 30:70v/v. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1 ml/min. Detection was carried out at 267 nm at ambient temperature.

Analytical Methodology

Preparation of Primary Standard Stock Solutions

Standard stock solutions were prepared by dissolving 50mg of Tolperisone and 40mg Etodolac working standard in two separate 100 ml and 50 ml volumetric flasks using 15ml of mobile phase and made up to the mark with mobile phase to obtain a final concentration of 500µg/mL and 400µg/mL of each TOL and ETD. From the above stock solutions, 5 and 10 ml aliquots each were pipette in to a 100mLvolumetric flask and dissolved in 25mL of the mobile phase and made up to the mark with the solvent to obtain a final concentration of 30µg/mL and 80µg/mL for Tolperisone and Etodolac respectively.

Preparation of Sample solutions

Twenty tablets were weighed and finely powdered. Accurately weighed and transferred equivalent to 150mg of TOL and 400mg of ETD into a 200ml volumetric flask, added 150 ml of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mixed. Filter the solution through 0.45 µm membrane Filter. Transferred 4.0ml of the above solution into a 100 ml volumetric flask and diluted to volume with diluent to obtain a concentration of 30µg/mL and 80µg/mL of TOL and ETD respectively.

Table 1: Chromatographic Conditions

Parameter	Content
Column	Sunsil, 250 mm x4.6 mm i.d; 5µ particle size
Mobile Phase	Acetonitrile and phosphate buffer (pH 2.6) in the ratio of 30:70 v/v
Flow Rate	1 ml/min
Run time	25 minutes
Temperature	Ambient
Injection Volume	20 µL
Detection & Wavelength	DAD detector, 267 nm
Retention times	2.957 & 4.193 minutes for TOL & ETD

Method optimization

Recommended Procedure

After systematic and detailed study of the various parameters involved as described under the results and discussion in this chapter, the following procedure was adopted for the determination of TOL and ETD in bulk samples and pharmaceutical formulations.

Procedure

Initially the mobile phase was pumped for about 30 minutes to saturate the column thereby to set the baseline corrected. Then 20 µL of the standard and sample solutions were injected separately. A quantitative determination of the active ingredients was made by comparison of the peak area of the sample injection with the corresponding peak area of the standard injection. The amount of TOL and ETD present in the sample were calculated through the standard calibration curve.

RESULTS AND DISCUSSIONS

The appropriate wavelength in the UV-region (267 nm), was selected for the measurement of the active ingredients in the proposed method. The

method was validated by linear fit curve and all the other parameters were calculated similar to the Spectrophotometric method and were discussed in the following pages. The typical chromatogram indicating the separation of TOL & ETD with Sunsil RP-C_{18} column (250 mm x4.6 mm i.d; 5µ particle size) and mobile phase consisting of Acetonitrile and phosphate buffer (pH 2.6) in the ratio of 30:70 v/v in gradient mode.

Table 2: Validation Summary / System Suitability

Parameter	Tolperisone HCl	Etodolac
Theoretical Plates (N)	5183	4143
Tailing factor	1.39	1.44
Retention time (min)	2.957	4.193
Resolution	------	5.82
% Peak Area	9.08	90.92
LOD (µg/mL)	1.30	1.88
LOQ (µg/mL)	3.93	5.70

Method validation

The following parameters were used to validate the method for the proposed assay procedure of TOL & ETD in pharmaceutical dosage forms. The developed HPLC method for the simultaneous determination of Thiocolchicoside and Aceclofenac was validated as per the ICHguidelines [8, 9].

Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e. method, system, Inter day and intraday. Precision of the developed method was assessed by measuring the response on the same day (intraday precision) and next two consecutive days (inter day precision). The precision of the method was assessed by six replicate injections of 100% test concentration. Intra and inter-day precision of the method was assessed by determination of standard deviation and % RSD for the analyte response. The result was given in Table 3.

Table 3: Method Precision (Inter and Intraday) studies for Tolperisone and Etodolac by proposed method

Summary showing Method Precision by Proposed Method			
Tolperisone HCl		**Etodolac**	
Method Precision (Inter &Intra Day)		Method Precision (Inter &Intra Day)	
99.1	100.1	98.9	98.6
99.2	100.5	98.6	98.4
99.5	99.5	98.6	98.6
99.4	99.7	98.1	98.1
98.5	98.9	98.5	98.9
99.4	98.4	98.8	98.4
Overall Avg.	99.35		98.52
Overage Std Dev.	0.66		0.40
Over all %RSD	0.66		0.41

Linearity and range

The standard curve was obtained in the concentration range of 18 - 42 µg/mL for TOL and 48 - 112µg/mL for ETD. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in Figure-3 **(Tolperisone HCl)** and **(Etodolac)** to demonstrate the linearity of the proposed method. The result of regression analysis was given above in the Table 4. Similarly the representative

chromatograms for linearity were shown in Figure: 4. from the data obtained which given in Table-4 (Tolperisone HCl & Etodolac) the method was found to be linear within the proposed range.

Table 4: Linearity studies for Tolperisone and Etodolac by proposed method

Linearity Study for Tolperisone			**Linearity Study for Etodolac**	
% Level	**Conc. µg/ml**	**Area**	**Conc. µg/ml**	**Area**
60	18.00	212.082	48.00	2124.641
80	24.00	284.081	64.00	2834.196
100	30.00	349.659	80.00	3456.154
120	36.00	413.469	96.00	4105.076
140	42.00	472.695	112.00	4759.097
Slope		10.8		40.874
Intercept		21.1		185.94
% Y-Intercept		194.5		454.9
Residual Sum of Squares		4.3		23.312
CC(r)		0.9994		0.9998
RSQ(r^2)		0.9987		0.9996
LOD		1.30		1.88
LOQ		3.93		5.70

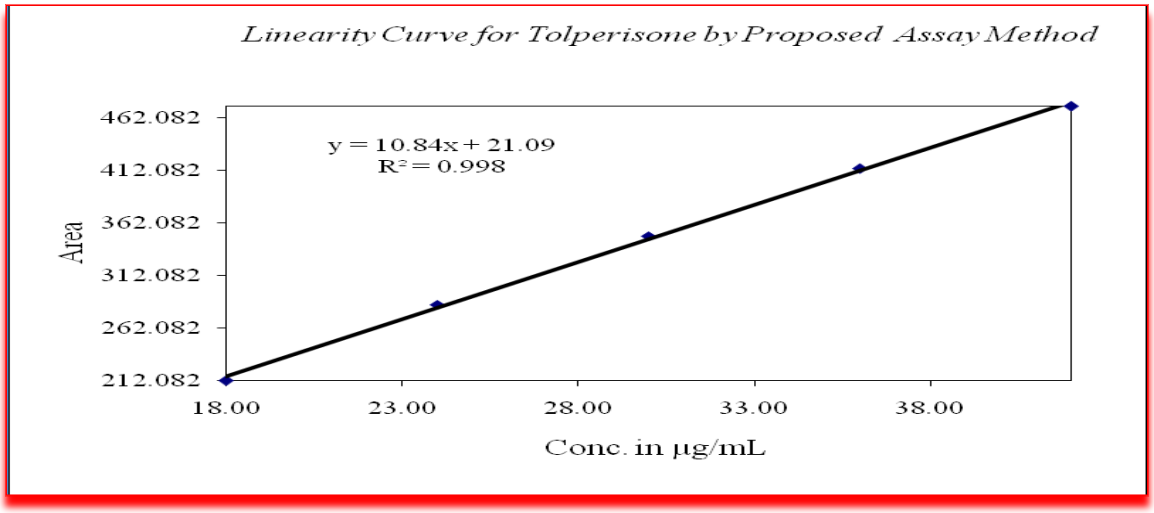

Figure 3: Calibration curve for Tolperisone

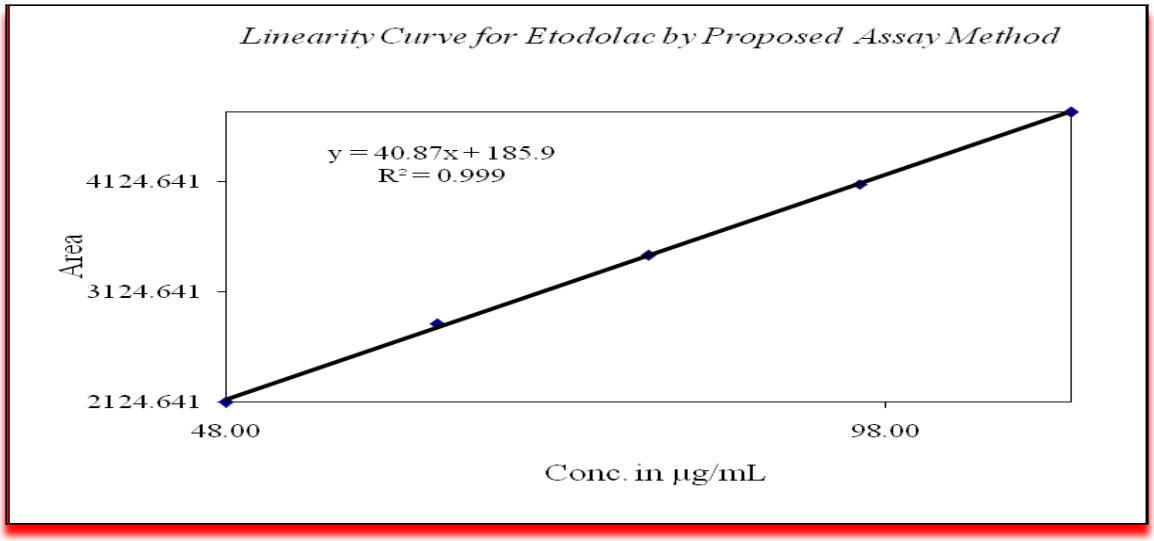

Figure 4: Calibration curve for Etodolac

System suitability

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 30 µg/mL of TOL and 80 µg/mL of ETD. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the below table 5.

Table 5: System suitability parameters for Tolperisone and Etodolac by proposed method

Name of the Compound	Retention Time	Theoretical plate	Tailing factor	USP Resolution
Tolperisone	2.957	1.39	5183	----
Etodolac	4.193	1.44	4143	5.82

Robustness

The robustness of the method was determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate. A deviation of ± 2 nm in the detection wavelength, ± 0.1 ml/min in the flow rate, ± 5% change in the organic phase were tried individually. The result was given in the Table 6.

Table 6: Robustness studies for Tolperisone and Etodolac by proposed method

Parameter		% RSD	
		Tolperisone	Etodolac
Wavelength (±2)	261 nm	0.22	0.36
	265 nm	0.34	0.57
Flow Rate (ml /min) (±0.2)	0.8 ml/min	0.68	0.49
	1.2 ml.min	0.54	0.31

Thermal Degradation Sample

Powders collected from 20 tablets are exposed to heat at 105°C for about 5 days. Accurately weigh and transfer equivalent to 150mg of Tolperisone and 400mg of Etodolac into a 200ml volumetric flask, add 150ml of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 4.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure: 5). Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

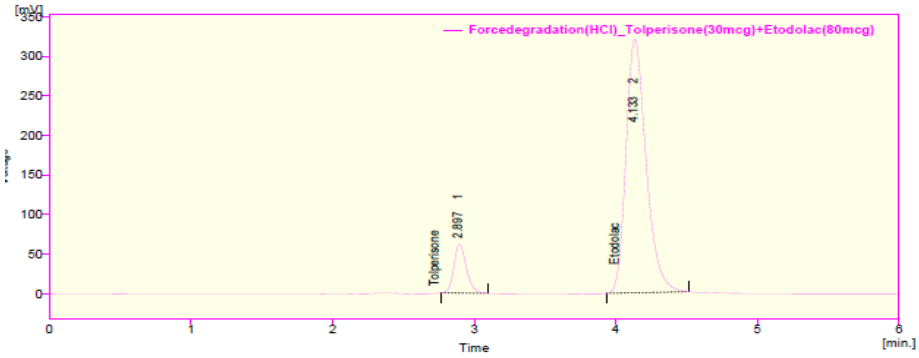

Figure 5: A typical HPLC Chromatogram showing the Control Sample profile of Tolperisone and Etodolac in Acidic hydrolysis by proposed method

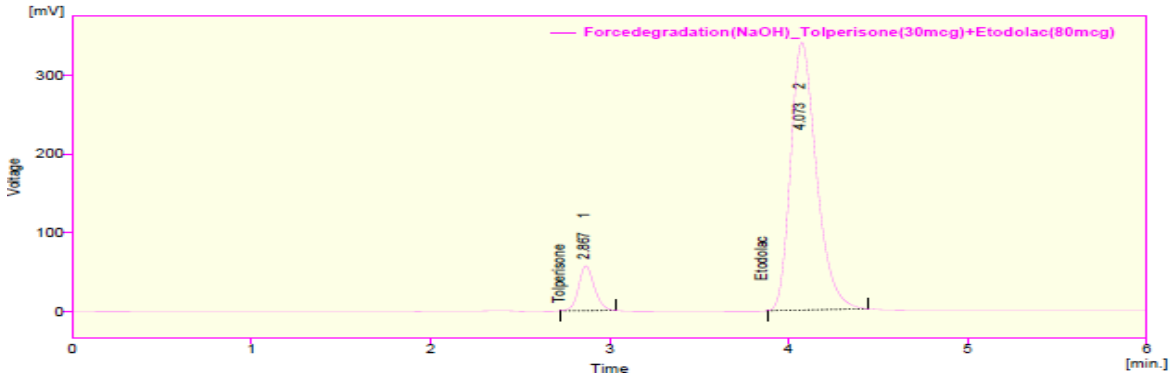

Figure 6: A typical HPLC Chromatogram showing the Control Sample profile of Tolperisone and Etodolac in Base hydrolysis by proposed method

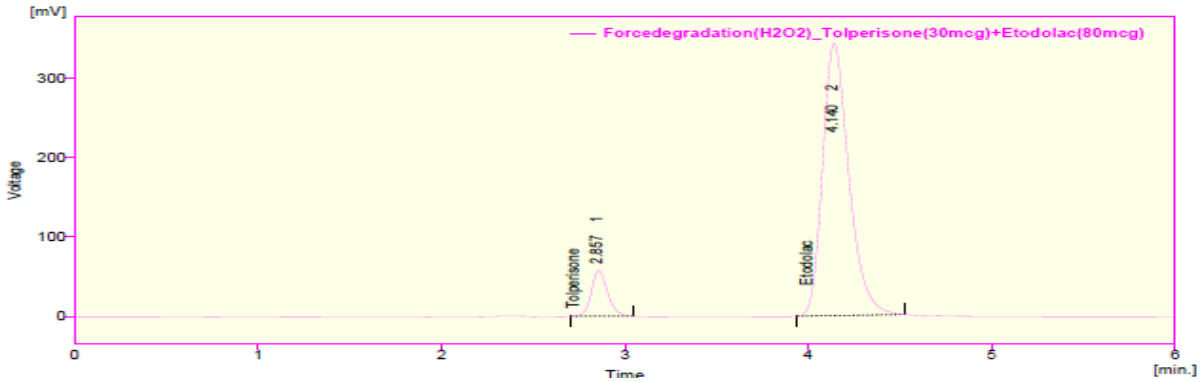

Figure 7: A typical HPLC Chromatogram showing the Control Sample profile of Tolperisone and Etodolac in Peroxide hydrolysis by proposed method

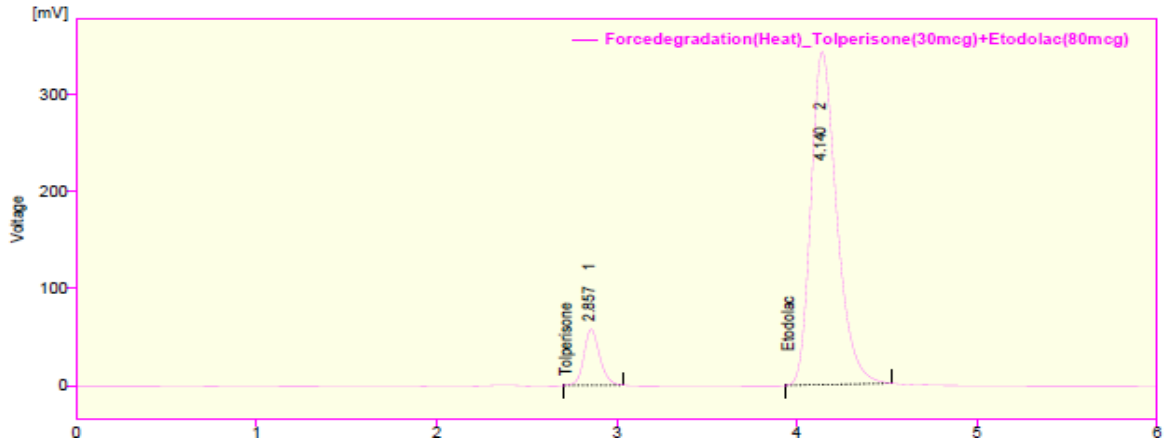

Figure 8: A typical HPLC Chromatogram showing the Control Sample profile of Tolperisone and Etodolac in Thermal hydrolysis by proposed method

Column chemistry, solvent selectivity, solvent strength (volume fraction of organic solvent(s) in the mobile phase), detection wavelength and flow rate were varied to determine the chromatographic conditions for giving the best separation. Several mobile phase compositions were tried to resolve the peaks of Tolperisone and Etodolac. The optimum results were attained with acetonitrile: phosphate buffer (pH 2.6) in the ratio 30:70 (v/v) because it could resolve the peaks of Tolperisone with retention time at 2.957 min and Etodolac retention time at 4.193 min. The two peaks were symmetric and sufficiently resolved. System suitability was carried out by injecting 5 replicate injections of 100 % concentration of Tolperisone and Etodolac. The resolution was found to be greater than 2.

Specificity of the chromatographic method was tested by injecting mobile phase as blank and sample concentration prepared from marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of Tolperisone and Etodolac at 2.957 min and 4.193 min respectively without any interference. Thus the developed method was specific for analyzing the commercial formulations for Tolperisone and Etodolac. The peak areas corresponding to the concentration range of Tolperisone 18-42 µg/mL and Etodolac 48-112 µg/mL prepared in triplicate were plotted against the respective concentrations. The calibration curves were linear in the range studied for Tolperisone and Etodolac, respectively, with mean correlation coefficients (n=3) of 0.999 and higher, Accuracy of the proposed method was assessed by standard addition method at 50 %, 100 % and 150 % levels of recovery to the pre analyzed sample in triplicate. The recovery of the added standard to the sample was calculated and it was found to be 97.8-99.8 % w/w for Tolperisone and 98.1-100.5 % w/w for Etodolac respectively and the % RSD was less than 2 for both the drugs which indicates good accuracy of the method.

LOD and LOQ were calculated from the average slope and standard deviation of Y intercepts of the calibration curve. Limit of Detection for Tolperisone and Etodolac were 1.30µg/mL and 1.88µg/mL respectively where as Limit of Quantitation of Tolperisone and Etodolac were 3.93µg/mL and 5.70µg/mL respectively indicating high sensitivity of the method. LOD and LOQ value was given in tables 7.6 – 7.7. The method is precise with a % RSD of less than 2 for both Tolperisone and Etodolac respectively. Robustness was carried out by change in the flow rate (±0.2 ml/min), mobile phase variation (±5%) and variation in wavelength (± 2 nm). Solution of 100 % concentration is prepared and injected in triplicate for each varied operational condition and % R.S.D was found to be less than 2. The proposed method was applied for the assay of commercial formulation containing Tolperisone and Etodolac. Each sample was analyzed in triplicate. The mean recovery values were 98.73 and 97.25 % for Tolperisone and Etodolac.

CONCLUSION

There is only a single report on the simultaneous HPLC determination of TOL & ETD in combination pharmaceutical formulations from the literature prior to the commencement of these investigations. The author has developed a sensitive, accurate and precise RP-HPLC procedure for the simultaneous estimation of TOL & ETD in bulk drug and also in pharmaceutical formulations.

The proposed RP-HPLC method for simultaneous assay TOL and ETD in combined dosage forms was validated, and it was found to be more applicable in various parameters discussed in this present chapter, which is more applicable for better routine quantitative analysis. In this HPLC method, the standard and sample preparations required less time and no tedious extraction were involved thereof. The low values of standard deviations are indicative of the high precision of the method developed. The results of linearity, precision, accuracy and specificity, were proved to be within the limits.

The absence of additional peaks in the chromatogram indicated non-interference of the common excipients used in the tablets. It is thus, demonstrated that the developed RP-HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can easily be used for the routine quality control of bulk and pharmaceutical formulations of TOL & ETD with a short analysis time.

It can be seen from the results presented that the proposed procedure has good precision and accuracy. The above proposed method obviates the need for any preliminary treatment and is simple, sensitive and reliable. Thus, the present procedures constitute a quick reported RP-HPLC method with good precision, accuracy and sensitivity for the simultaneous estimation of TOL & ETD in pure stage and also in combination products.

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